Urea metabolism in beef steers grazing Bermudagrass, Caucasian bluestem, or gamagrass pastures varying in plant morphology, protein content, and protein composition

G. B. Huntington, J. C. Burns and S. L. Archibeque

J Anim Sci 2007.85:1997-2004.
doi: 10.2527/jas.2006-597 originally published online Apr 12, 2007;

Urea metabolism in beef steers grazing Bermudagrass, Caucasian bluestem, or gamagrass pastures varying in plant morphology, protein content, and protein composition[1,2,3,4]

G. B. Huntington,*[5] J. C. Burns,*†‡ and S. L. Archibeque*[6]

*Animal Science Department, and †USDA-ARS, ‡Crop Science Department, North Carolina State University, Raleigh 27695

ABSTRACT: Pastures of Bermudagrass (*Cynodon dactylon,* BG), Caucasian bluestem (*Bothriochloa caucasica,* CBS), and gamagrass (*Tripsacum dactyloides,* GG) were evaluated from the perspectives of forage composition, selection during grazing, and N metabolism in beef steers. All pastures were fertilized with 78 kg/ha of N approximately 60 and 30 d before sample collection. In 2000 and 2001, 12 steers (250 kg of BW) were blocked based on BW and then assigned randomly to a replicated, randomized complete block design, with 2 pastures of each forage and 2 steers per pasture. Three other steers with esophageal fistulas were used to collect masticate samples to represent intake preferences. Herbage mass was >1,900 kg/ha. After at least 14 d of adaptation, urine and blood samples were collected for determination of serum urea N and percentage of urinary N in the form of urea. One steer per pasture (6 steers per year) was infused i.v. with ^{15,15}N urea for 50 h before collecting urine for 6 h to measure urea N enrichment, urea entry rate, urinary urea excretion, gut urea recycling, and return of urea N to the ornithine cycle. The canopy leaf:stem DM ratio differed (P = 0.01) among BG (0.50), CBS (1.01), and GG (4.00). Caucasian bluestem had less CP (% of DM) than GG or BG in the canopy (9.6 vs. 12.0 or 12.3, P = 0.07) and in the masticate (9.8 vs. 14.7 or 13.9, P = 0.04). Bermudagrass had less true protein (% of CP) than CBS or GG in the canopy (72.9 vs. 83.3 or 83.0, P = 0.07) and in the masticate (73.7 vs. 85.8 or 88.0, P = 0.04). Compared with GG and BG, CBS had less serum urea N (10.1 or 12.2 vs. 2.5 mM, P = 0.01), urea entry rate (353 or 391 vs. 209 mmol of N/h, P = 0.07), and urinary urea excretion (105 or 95 vs. 18 mmol of N/h, P = 0.04), and a greater return of urea N to the ornithine cycle as a proportion of gut urea recycling (0.109 or 0.118 vs. 0.231, P = 0.02). Urea production and recycling in these steers responded more to the N concentration in the grasses than to differences in plant protein fractions. There was no evidence of improved N capture by the steers due to changes in the leaf:stem ratio among the grasses at the herbage mass evaluated.

Key words: beef cattle, urea biosynthesis, *Tripsacum dactyloides*, *Cynodon dactylon*, *Bothriochloa caucasica*

J. Anim. Sci. 2007. 85:1997–2004
doi:10.2527/jas.2006-597

INTRODUCTION

Monitoring endogenous urea production in ruminants is a way to evaluate N metabolism (Nolan, 1975; Kennedy and Milligan, 1980; Huntington and Archibeque, 1999). In general, urea production and excretion are inversely related to efficient capture of dietary N as animal growth or product (Huntington and Archibeque, 1999, Lobley et al., 2000). Voluntary selection by grazing ruminants or supplementation that improves synchrony between N and carbohydrate fermentation in the rumen (Bach et al., 1995) should minimize urea production, enhance its recycling to the gut, improve N use by ruminants, and reduce N excretion into the environment.

[1]This research was supported in part by the North Carolina Institute of Nutrition.

[2]The authors thank Lucie Smith, Tina Starr, Vicki Fouts, and Roxane Fagan for their able technical support of this research.

[3]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service or USDA-ARS, of the product named or criticism of similar items not mentioned.

[4]All programs and services of the USDA are offered on a nondiscriminatory basis with regard to race, color, national origin, sex, age, marital status, or handicap.

[5]Corresponding author: Gerald_Huntington@ncsu.edu

[6]Current address: Department of Animal Science, Colorado State University, Fort Collins.

Received September 5, 2006.
Accepted April 5, 2007.

Total N and N distributed in the leaf and stem fractions are grass-specific and change in response to factors like fertilization rate and harvest date (Archibeque et al., 2001; Johnson et al., 2001), maturity at harvest (Elizalde et al., 1999; Alzueta et al., 2001), and postharvest processing (Agbossamey et al., 1998; Archibeque et al., 2002). Archibeque et al. (2002) reported interactions between urea N entry rate in steers and intake of protein fractions from 3 grass hays.

Grazing behavior (Hodgson et al., 1994) and ADG of beef steers are related to differences in the leaf:stem ratio of the forage species. For example, in continuously stocked pastures, the average leaf:stem ratio of grasses and the ADG of steers was 0.4 and 0.3 kg, respectively, for Tifton 44 Bermudagrass vs. 2.4 and 0.8 kg, respectively, for Pete gamagrass (Burns et al., 1992).

We hypothesized that morphological and chemical differences in our available stands of Caucasian bluestem (**CBS**), Bermudagrass (**BG**), and gamagrass (**GG**) would change urea metabolism in grazing steers. Our objectives were to measure urea production and recycling in grazing beef steers and to evaluate 3 warm-season perennial grasses (GG, BG, and CBS) with canopies providing a wide range in the leaf:stem ratio.

MATERIALS AND METHODS

Animals and Treatments

The experiment was conducted under the supervision and approval of our Institutional Animal Care and Use Committee.

During 2000 (yr 1) and 2001 (yr 2), replicated, well-established pastures of Tifton 44 BG (*Cynodon dactylon* L.), CBS (*Bothriochloa caucasica* [Trin.] C.E. Hubb), and Iuka GG (*Tripsacum dactyloides* L.) were topdressed with 78 kg/ha of N as ammonium nitrate approximately 60 and 30 d before sample collection, respectively. Maintenance fertilizer (P and K) and lime were applied in the spring based on the soil test. The pastures were initially stocked in the spring when the canopy height reached about 0.4 m for GG and 0.2 m for BG and CBS. Pastures were 0.4 ha for GG and CBS and 0.2 ha for BG; each was equipped with shade and water. Trace-mineralized salt was available free choice. Additional steers were used at the onset of grazing with variable, periodic stocking to maintain proper herbage mass for each species and to maintain approximately equal herbage mass within species. Extra steers were removed when the experimental steers were allocated to the forage treatments 14 d before initiation of the experimental period. Each year, 12 experimental steers (medium-frame, black haired; 239 ± 11 kg of BW in yr 1 and 264 ± 21 kg of BW in yr 2) were blocked on the basis of BW, and then 2 steers were assigned randomly to each pasture in a randomized complete block design, with 2 pasture replicates.

Herbage Mass Characterization

Herbage mass (kg of DM/ha) was measured the day before intravenous infusion of ^{15}N urea into the steers. This was achieved by cutting 8 random 0.24-m^2 quadrats from each pasture. The stubble height was species specific because of differences among morphology of the grasses: 20 mm for BG and CBS and 55 mm for GG. The herbage from the 8 quadrats was pooled and weighed, and 1 subsample was taken for DM determination and another was frozen in liquid N_2 and stored (–15°C) until freeze-dried. In yr 2 the pasture canopy was further characterized for the proportion of the DM that consisted of leaf blade (hereafter referred to as leaf), stem plus sheath (hereafter referred to as stem), and seed heads, dead material, weeds, and uncharacterized material (hereafter referred to as other). The canopy samples were cut, bagged in the field to retain their integrity, transported to the laboratory, and refrigerated for subsequent separation. The canopy fractions were then frozen, freeze-dried, and all herbage mass and canopy fraction samples were ground to pass a 1-mm screen and stored (–15°C) for laboratory analyses. Samples in yr 1 were ground through a cyclone mill (UDY Corp., Ft. Collins, CO), and samples in yr 2 were ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ).

Masticate Collection

Three Hereford steers (844 kg of BW) fitted with esophageal cannulas (Ellis et al., 1984) were used for estimating diet selection. Steers were assigned randomly to treatments, and masticate samples were collected on 2 consecutive days from approximately 0600 to 0900 each day. On d 2, the steers were rotated to the next pasture treatment so that 2 different steers sampled each pasture, and the data from the steers were averaged. At sampling, the cannula was removed, the first 5 to 7 boluses were discarded, and masticate was collected over 30 min in a butterfly net lined with plastic. The samples (including saliva) were immediately transferred to plastic bags and frozen in liquid N_2. After quick-freezing in the field, the masticate samples were stored (–15°C) until freeze-dried. The dried whole-masticate samples were then ground in a Wiley mill to pass a 1-mm screen and returned to the freezer until laboratory analyses.

Urea Infusion and Sample Collection

Before the i.v. infusion of ^{15,15}N urea, each of the 12 steers was weighed and a urine sample was collected from each steer. Urine was collected in a plastic bag attached to a rubber ring that was strapped with elastic around the midsection of the steer. A jugular catheter was installed in 1 of the 2 steers assigned to each pasture replicate (6 steers each year), and a blood sample was collected from the catheter of each steer. After blood sampling, the apparatus containing the infusion pump

(P625, Instech Laboratories Inc., Plymouth Meeting, PA) was fitted to the catheterized steers, and an i.v. infusion of ^{15,15}N urea (Cambridge Isotope Laboratories, Andover, MA) in sterile, physiological saline (0.15 *M* NaCl) was begun. All steers were returned to their assigned pastures and remained there during the isotope infusion, except for periodic checks for proper function of the pumps and for replenishment of the infusate.

The infusion apparatus consisted of a plastic box (19 × 16 × 11 cm) with a hinged lid attached to a flap of leather that was attached to a leather halter. The plastic box contained the infusion pump, a battery, and a 500-mL bag of saline solution. A sterile tube passed through the head of the peristaltic infusion pump and connected the saline solution to the jugular catheter. The apparatus, including the halter and saline bag, weighed approximately 2.5 kg. The plastic box resided caudal to the poll on the steer's neck. The infused solution contained 15.0 m*M* ^{15,15}N urea in yr 1 and 15.7 m*M* ^{15,15}N urea in yr 2. The infusion rate for each pump was measured before and after the experiment. The average infusion rate was 0.266 mL/min in yr 1 and 0.283 mL/min in yr 2. The bag containing the ^{15,15}N urea solution was replenished once during the first 34 h of infusion, and then a different bag was used without replenishment for the remainder of the infusion.

To allow asymptotic enrichment of urea N (Lobley et al., 2000; Archibeque et al., 2002), urine samples were collected after approximately 50 h of infusion, with the collection apparatus described previously at hourly intervals until at least 3 samples were collected for each steer, or until 56 h of infusion had elapsed. Urine was removed from each steer's apparatus after each urination. A 25-mL sample was removed from the first urination at an hourly interval and acidified to pH < 4, and the remaining urine was transferred to a plastic container to make urine composites within steer. Subsequent urinations within the hour were added to the composite container. At the end of collection, all urine collected was acidified to pH < 4, weight of the urine was recorded, and a sample of the urine composite was retained. At the end of the infusion, each steer was weighed, and another blood sample was collected before the jugular catheter was removed from the catheterized steers. One blood (jugular venipuncture) and one urine sample were collected from the other steer in each pasture replicate (6 steers total) during the last 5 h of the 56-h infusion.

Isolation of Urea from Urine and Determination of N Enrichment

Urea was separated from ammonia or AA in the urine with cation exchange columns, as described by Archibeque et al. (2001). Recovery of known amounts of urea by this procedure ranged from 40 to 75%; recovery of 50% of added urea was assumed in calculating the volume of urine needed to recover from 50 to 70 μg of urea N for determination of N enrichment. Because the urea N concentration in the urine samples ranged from 5 to 600 m*M*, the volume of urine placed on the columns ranged from 0.25 to 4.0 mL. Water was added to all urine samples to make 5 mL of total volume placed on the columns. Samples were analyzed in duplicate. Eluate (20 mL) from each column was collected in a 50-mL beaker; water was removed by drying overnight at 60°C; the sample was reconstituted in 3 mL of 0.1 *M* phosphate buffer, pH 7.0, and then stored frozen until further analysis. The amount of sample used in subsequent analysis was varied to deliver the desired amount of urea N. The methods described by Marini and Attene-Ramos (2006) were used to generate $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ from monomolecular reactions of urea in the samples, and the enrichment of those gases was quantified with a DELTA^{plus}, single inlet, mass spectrometer (Thermo Finnigan, San Jose, CA).

Other Analytical Procedures

Archibeque et al. (2001) and Licitra et al. (1996) described and cited procedures used to determine N concentrations and CP fractions of the canopy and masticate samples. The CP fractions are separated by chemical procedures. Briefly, the CP fractions are nonprotein N (fraction A), 3 true protein fractions that decrease in ruminal degradability (fractions B1, B2, and B3), and ADF-N (fraction C). Urea N content of serum and urine was analyzed using the diacetyl monoxime method of Marsh et al. (1957).

Calculations and Statistical Analyses

The GLM procedure (SAS Inst. Inc., Cary, NC) was used to analyze the ^{15}N enrichment data, to test for differences in enrichment of $^{29}N_2$ or $^{30}N_2$ among sampling times, to test for differences between enrichment of $^{29}N_2$ or $^{30}N_2$ of individual urine samples and pooled urine samples within steers and to calculate the deviation of enrichment of $^{29}N_2$ or $^{30}N_2$ from the mean of the individual times. Sample time did not affect ($0.99 > P > 0.17$) enrichment of $^{29}N_2$, except for 1 steer grazing CBS ($P = 0.09$) and 1 steer grazing GG ($P = 0.08$) in yr 1. Sample time did not affect ($0.83 > P > 0.17$) enrichment of $^{30}N_2$, except for 1 steer grazing CBS in yr 1 ($P = 0.01$). There were no differences ($0.84 > P > 0.15$) between enrichment of $^{29}N_2$ or $^{30}N_2$ within steers, except for 1 steer grazing GG in yr 1 and 2 ($P < 0.02$). Within treatments, individual urine samples or pooled urine samples within steers did not differ in enrichment of $^{29}N_2$ ($P = 0.57$) or $^{30}N_2$ ($P = 0.56$). Across steers, means ± SED of the paired *t*-test difference in enrichment of $^{29}N_2$ between individual urine samples and pooled urine was –0.002 ± 0.0009 atoms percent excess, and for $^{30}N_2$ the difference between individual urine samples and pooled urine was –0.008 ± 0.003 atoms percent excess. Within steers, enrichment of $^{29}N_2$ ranged from –0.020 to 0.013 of the steer's mean of sample times, and enrichment of $^{30}N_2$ ranged from –0.032 to 0.038 atoms percent

excess of the steer's mean of sample times. Paired differences of the average of individual samples minus the pooled sample within steers of urea N entry rate was 32 ± 8 mmol/h.

Because of the small, and largely statistically nonsignificant, differences in data derived from individual urine samples and pooled urine samples, and because the urinary urea N excretion (mmol/h) was calculated from pooled urine samples, we calculated urea N entry rate by dividing the infusion rate of ^{15}N urea by the urine enrichment of $^{30}N_2$ in pooled urine samples (Lobley et al., 2000). The amount of ^{15}N urea infused (<0.3 mmol/h) was considered to be negligible and was not considered in calculation of urea N entry rate. Gut urea N recycling (mmol/h) was calculated as the difference between urea N entry rate and urinary urea N excretion, and return of urea N to the urea cycle (mmol/h) was calculated by multiplying the gut urea N recycling by the proportion of total enrichment as $^{29}N_2$ by gut urea N recycling divide by the urea N entry rate (Lobley et al., 2000).

The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to test for differences among treatments in the forage or masticate composition, and for treatment differences in parameters of N metabolism. The model included treatment as a fixed effect; year, the year × treatment interaction, and pastures within treatments were random effects. The model for the leaf:stem ratio and CP composition included treatment as a fixed effect, and pastures within treatments were random effects. Pasture was the experimental unit, and so the data for serum urea N and the percentage of urine N in the form of urea N were averaged for the 2 steers within pasture and year. Our criteria for type I error was $P < 0.10$. If the treatment effect was significant, treatment means were compared with Student's protected *t*-test. Simple correlation analysis across treatments indicated linear relationships among masticate components and measures of urea metabolism. Regression parameters of urea N entry rate and urinary urea N excretion as functions of masticate protein components were calculated with the GLM procedure of SAS. The analysis contained grass species as a class variable, the masticate component as a covariate, and the grass species × masticate component interaction (St-Pierre, 2001).

RESULTS

Pastures were stocked so that forage was not limiting during the experimental periods. Canopy heights (80 readings per pasture) averaged 0.24, 0.29, and 0.43 m for BG, CBS, and GG, respectively. This resulted in herbage mass averaging 2,878 kg/ha for BG (2,697 kg/ha in yr 1 and 3,059 kg/ha in yr 2), 2,720 kg/ha for CBS (2,390 kg/ha in yr 1 and 3,056 kg/ha in yr 2), and 2,078 kg/ha for GG (1,972 kg/ha in yr 1 and 2,183 kg/ha in yr 2). The trend ($P = 0.16$) for less herbage mass for GG compared with the other forages is, in part, attributable to characteristically less dense stands for GG compared with the other grasses and to the higher stubble for GG at harvesting and, consequently, the proportionally greater residue required to avoid stand loss. The greater canopy height for GG, compared with the other grasses, assured adequate available pasture at all times for all steers during the trial.

Table 1. Morphology, leaf:stem ratio, and CP composition of the canopy for Caucasian bluestem (CBS), Bermudagrass (BG), and gamagrass (GG) in yr 2

	Grass			
Item	CBS	BG	GG	SEM[1]
Plant part DM, % of total DM				
Leaf	44.0[c]	25.0[d]	72.0[e]	0.03
Stem	44.0[c]	50.0[d]	18.0[e]	0.03
Other[2]	12.0[acd]	25.0[bc]	10.0[ad]	0.03
Leaf:stem DM	1.01[c]	0.50[d]	4.00[e]	0.03
CP, % of DM in each fraction				
Leaf	12.2[c]	18.5[d]	14.1[c]	0.8
Stem	7.9[a]	10.1[b]	9.7[ab]	0.6
Other[2]	12.1	10.1	13.7	1.6
Leaf:stem CP	1.54[acd]	1.84[bc]	1.46[abd]	0.07

[a,b]Within rows, means without common superscripts differ ($0.05 < P < 0.10$).
[c–e]Within rows, means without common superscripts differ ($P < 0.05$).
[1]n = 2.
[2]Includes seed heads, dead material, and weeds.

Canopy morphology varied as expected among grasses (Table 1). Gamagrass had the greatest ($P = 0.05$) percentage of leaf and least ($P = 0.05$) percentage of stem, BG had the least ($P = 0.05$) percentage of leaf and greatest ($P = 0.05$) percentage of stem, and CBS was intermediate between GG and BG in those characteristics. Bermudagrass had greater ($P = 0.05$) CP (% of leaf DM) than CBS or GG, and greater ($P = 0.09$) CP in the stem (% of DM) than CBS (Table 1). Caucasian blue stem had less CP (% of DM) in canopy ($P < 0.10$) and masticate ($P = 0.05$) than the other grasses (Table 2). Fraction B3 (% of CP) in canopy ($P = 0.10$) and masticate ($P = 0.05$) was greatest in CBS, least in BG, with GG intermediate. Bermudagrass had greater ($P < 0.10$) concentration of NPN and less ($P < 0.10$) concentration of true protein in canopy and masticate, and greater ($P < 0.10$) concentration of fraction A in canopy than the other 2 grasses (Table 2). Grasses did not differ ($0.16 < P < 0.59$) in fractions B1, B2, or C. Paired comparisons of masticate and canopy samples (data not shown) indicated greater ($P < 0.01$) concentrations in masticate of CP (% of DM) for BG ($P = 0.09$) and GG ($P = 0.09$), greater concentration of true protein (% of DM) for GG ($P = 0.06$), and greater ($P = 0.09$) concentration of fraction B1 (% of CP) for GG.

Steers grazing CBS had less serum urea N ($P = 0.01$), urea N as a percentage of total urinary N ($P = 0.01$), urea N entry rate ($P = 0.07$), urinary urea N excretion ($P = 0.05$), and urinary urea N excretion divided by urea N entry rate ($P = 0.02$) than steers grazing GG or BG (Table 3). Conversely, steers grazing CBS had

Table 2. Protein fractions in the canopy and masticate samples of Caucasian bluestem (CBS), Bermudagrass (BG), and gamagrass (GG)

	Grass			
Item	CBS	BG	GG	SEM[1]
% of canopy DM				
CP	9.64[a]	12.34[b]	12.05[b]	0.80
True protein	8.03[a]	8.97[ab]	10.00[b]	0.83
NPN	1.60[a]	3.37[b]	2.04[a]	0.25
% of masticate DM				
CP	10.38[c]	13.94[d]	14.73[d]	0.93
True protein	8.91[c]	10.28[d]	12.98[d]	1.09
NPN	1.84[a]	3.66[b]	1.74[a]	0.46
Protein fraction,[2] % of canopy CP				
True protein	83.30[a]	72.86[b]	82.98[a]	2.06
A	16.70[a]	27.14[b]	17.02[a]	2.06
B1	9.22	7.39	8.39	1.11
B2	28.14	27.75	32.07	2.28
B3	42.88[a]	33.76[b]	39.05[ab]	1.49
C	3.07	3.97	3.47	0.28
Protein fraction,[2] % of masticate CP				
True protein	85.79[c]	73.70[d]	87.98[c]	3.04
A	18.33[ab]	26.30[a]	12.02[b]	4.49
B1	11.11	11.21	13.22	1.78
B2	25.99	27.08	30.99	4.22
B3	45.58[c]	31.26[d]	40.34[cd]	3.43
C	3.11	4.15	3.43	0.64

[a,b]Within rows, grasses without common superscripts differ ($0.05 < P < 0.10$).
[c,d]Within rows, grasses without common superscripts differ ($P < 0.05$).
[1]n = 2 for canopy and masticate protein fractions. Within the 2 replicates (years), there are 2 observations for canopy and 4 observations for masticate protein fractions.
[2]Fraction A is nonprotein N, fractions B1, B2, and B3 are protein fractions with decreasing ruminal degradability, and fraction C is ADF-N (Licitra et al., 1996).

greater gut urea N recycling divided by urea N entry rate ($P = 0.02$) and return of urea N to the ornithine cycle divided by gut urea N recycling ($P = 0.01$) than steers grazing GG or BG. Treatments did not differ in return of urea N to the ornithine cycle ($P = 0.33$). However, the rates of gut urea N recycling for steers grazing BG or GG were greater ($P = 0.07$) than the rate for steers grazing CBS. Other than small changes in experimental means, ANOVA of urea kinetic data from the average of individual samples within steers provided the same statistical inferences among treatments as those in Table 3, which were from pooled samples within steers, except gut urea N recycling was less ($P = 0.05$) for CBS than for the other grasses.

DISCUSSION

Urea production and recycling have been measured with confined sheep (Lobley et al., 2000) and steers (Archibeque et al., 2001, 2002). Free-ranging ruminants present special research challenges, including accurate measures of intake, urine excretion, and endogenous urea production. Our results are limited by the absence of measures of intake, but the ability to relate urea metabolism to masticate samples in our view improves interpretation of results. Protein fractions of canopy and masticate (Table 2) rank the 3 grasses in the same order, except for CP (% of DM). In addition, replication for 2 consecutive years adds variation and biological relevance to the results. We did not collect urine for 24 h, but we were successful in direct urine collection for 6 h from grazing animals and know of no similar published data. We accomplished this by training the steers to be led by halter, to accept approach by humans in the pasture, and by attaching a 3-m rope to the steers' halters.

We expected a range in leaf:stem in the grasses that we compared. Burns et al. (1992) reported leaf:stem of 2.4:1 for GG and 0.36:1 for BG. We know of no published leaf:stem data for CBS, but unpublished data indicated CBS likely is intermediate between the other 2 grasses, as we found in our samples (Table 1). Grazing steers select for leaf over stem (Hodgson et al., 1994). Burns et al. (1992) found similar in vitro DM digestibilities for leaf and stem components of GG and BG. They also found less dead material in GG than in BG, similar to our data for the other fraction (Table 1), which was composed mainly of dead material. Finally, we expected differences in protein fractions between GG and BG; based on data of Johnson et al. (2001), Archibeque et al. (2001), and Magee (2004), we expected GG to have greater concentrations of fraction B3 and lesser concentrations of fractions A and B1 than BG. We know of no published protein fraction data for CBS.

Archibeque et al. (2002) reported interactions between urea N entry rate and intake of fractions B2 or

Table 3. Steer BW and urea metabolic responses to grazing Caucasian bluestem (CBS), Bermudagrass (BG), and gamagrass (GG) pastures

	Grass			
Item	CBS	BG	GG	SEM[1]
BW, kg	256	249	250	14
Serum urea N, m*M*	2.49[a]	12.23[b]	10.14[b]	0.70
Urinary urea N, % of urine N	31.5[a]	69.9[b]	66.3[b]	9.3
Urea entry rate (UER), mmol of N/h	209[a]	391[b]	354[b]	39
Urinary urea excretion (UUE), mmol of N/h	19[a]	95[b]	105[b]	16
Gut urea recycling (GUR), mmol of N/h	191[c]	297[d]	249[cd]	29
UUE/UER	0.087[a]	0.246[b]	0.293[b]	0.024
GUR/UER	0.913[a]	0.754[b]	0.702[b]	0.024
Return to the ornithine cycle (ROC), mmol of N/h	46	37	28	6.6
ROC/GUR	0.239[a]	0.122[b]	0.113[b]	0.015

[a,b]Within rows, grasses with different superscripts differ ($P < 0.05$).
[c,d]Within rows, grasses with different superscripts differ ($P = 0.07$).
[1]n = 4.

B3 from 3 grass hays. They found that the increase in urea N entry rate of steers fed GG and switchgrass was greater than urea N entry rate of steers fed tall fescue in response to increased intake of fraction B2. The steers' urea N entry rate was positively related to fraction B3 intake from GG or switchgrass, but inversely related to B3 intake from tall fescue. We did not measure intake in our experiment, but there were simple correlations across treatments between urea N entry rate and CP of masticate ($P = 0.01$, r = 0.83), between urinary urea N excretion and CP of masticate ($P = 0.01$, r = 0.60), between urea N entry rate and true protein of masticate ($P = 0.03$, r = 0.61), and between urea N entry rate and B3 fraction of masticate ($P = 0.06$, r = –0.56). Pooled regression of urea N entry rate and urinary urea N excretion for replicates, as a function of CP concentration in masticate (St-Pierre, 2001; Figure 1), shows an expected increase in urea N entry rate as CP concentration increases, presuming similar DMI among grasses. The area between the regression lines for urea N entry rate and urinary urea N excretion in Figure 1 represents increased predicted recycling of urea N to the gut as CP concentration in the masticate increased, which is consistent with the treatment means for CP concentrations in canopy (Table 2) and gut urea N recycling (Table 3). The pooled regression of urea N entry rate on true protein concentrations in masticate (Figure 2) was similar to the response to CP shown in Figure 1, although true protein concentration of BG in masticate was numerically closer to CBS than GG. Protein fraction B3 represents true protein of limited degradability in the rumen, but available for postruminal digestion and absorption (Licitra et al., 1996). Regression of urea N entry rate as a function of fraction B3 expressed as a percentage of DM (Figure 3) or as a percentage of CP (Figure 4) shows similar, positive responses to increased protein concentration as that seen for CP (Figure 1) and true protein (Figure 2). We interpret the apparent discrepancy of B3 (% of CP) for BG (Figure 4) to be a function of variation among steers within replicates that had similar concentrations of B3.

We expected that greater leaf:stem for GG vs. BG would be linked mechanistically to steers' preference for leaf vs. stem (Hodgson et al., 1994), a greater ruminal supply of CP and fermentable DM (Fisher et al., 1991; Burns et al., 1992), less ruminal degradation of dietary protein, and more incorporation of NPN into microbial protein (Kennedy and Milligan, 1980; Huntington and Archibeque, 1999). We also expected to detect urea kinetic parameters and serum urea N concentrations that indicated steers grazing GG were more efficient than steers grazing BG in retaining similar supplies of di-

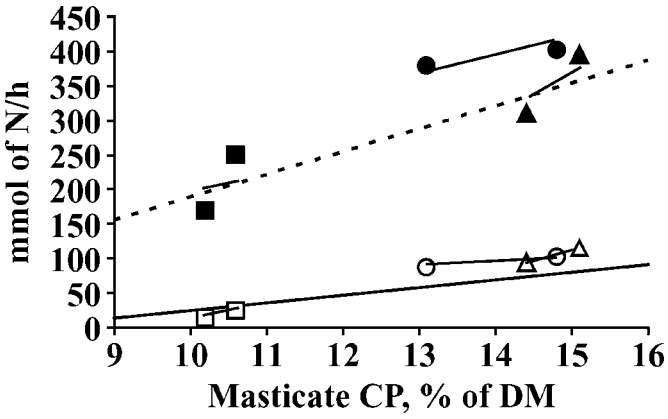

Figure 1. Urea entry rate (UER, shaded symbols) and urinary urea excretion (UUE, open symbols) in response to varying levels of CP in masticate of steers grazing Caucasian bluestem (■), Bermudagrass (●), or gamagrass (▲). Each point is the mean of a replicate (pasture) across years, lines between points are regressions within grasses, and the lines extending across the plot area are the pooled regressions (St-Pierre, 2001). Pooled regression for UER = (32.8 × CP, % of DM) – 141, $r^2 = 0.82$, Pooled regression for UUE = (11 × CP, % of DM) – 85, $r^2 = 0.90$.

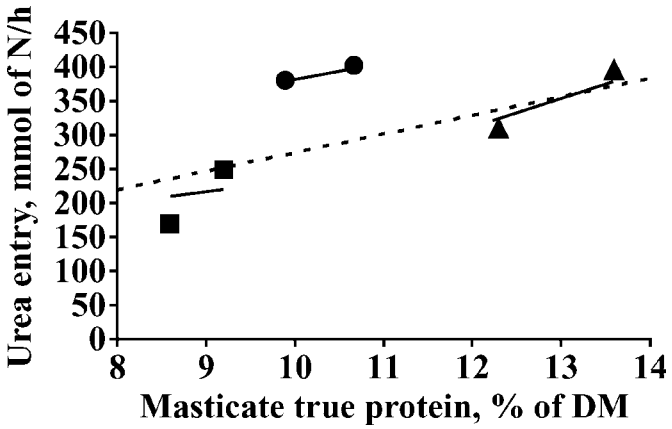

Figure 2. Urea entry rate (UER) in response to varying levels of true protein in masticate of steers grazing Caucasian bluestem (■), Bermudagrass (●), or gamagrass (▲). Each point is the mean of a replicate (pasture) across years, lines between points are regressions within grasses, and the line extending across the plot area is the pooled regression (St-Pierre, 2001). Pooled regression for UER = (27.4 × true protein, % of DM) − 1, r^2 = 0.83.

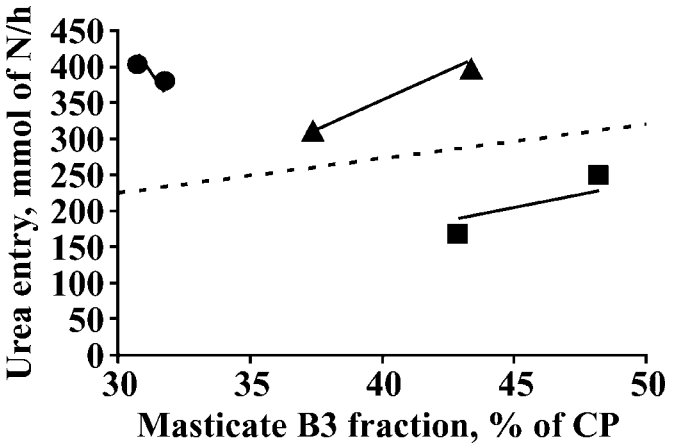

Figure 4. Urea entry rate (UER) in response to varying levels of B3 protein fraction (% of CP) in masticate of steers grazing Caucasian bluestem (■), Bermudagrass (●), or gamagrass (▲). Each point is the mean of a replicate (pasture) across years, lines between points are regressions within grasses, and the line extending across the plot area is the pooled regression (St-Pierre, 2001). Pooled regression for UER = (4.76 × B3, % of CP) + 81, r^2 = 0.77.

etary N for growth because Burns et al. (1992) reported greater ADG for steers grazing GG compared with BG. In spite of greater (P = 0.05) leaf:stem DM ratio (8 times greater for GG vs. BG, Table 1), greater true protein (P = 0.10), and less (P = 0.10) fraction A in the masticate from GG vs. BG (Table 2), no associated changes in serum urea N, urea N entry, urinary excretion, gut recycling, or return to the ornithine cycle were detected (Table 3). Few observations (4 steers per treatment) and variability attributable to replication over 2 yr may explain in part the lack of association.

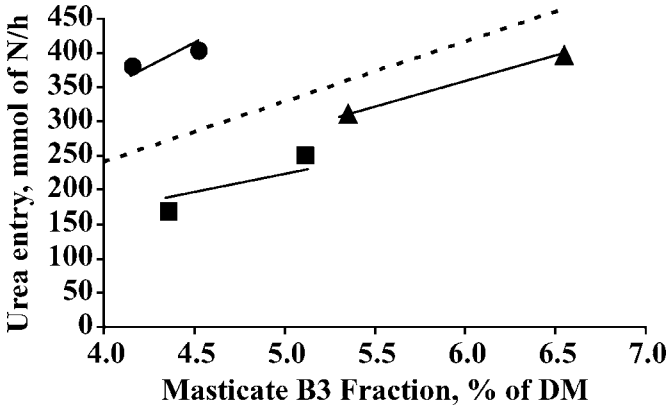

Figure 3. Urea entry rate (UER) in response to varying levels of B3 protein fraction (% of DM) in masticate of steers grazing Caucasian bluestem (■), Bermudagrass (●), or gamagrass (▲). Each point is the mean of a replicate (pasture) across years, lines between points are regressions within grasses, and the line extending across the plot area is the pooled regression (St-Pierre, 2001). Pooled regression for UER = (88.2 × B3, % of DM) − 113, r^2 = 0.86.

The CP concentration of the grasses (Table 2) and serum urea N concentrations (Table 3) indicate that steers grazing CBS responded to less N supply by decreasing urea N entry rate, by recycling a greater proportion of urea N entry to the gut, and by returning a greater portion of gut urea N recycling to the ornithine cycle (Table 3). However, the rates of gut urea N recycling and rates of return of recycled urea N to the ornithine cycle did not differ significantly among grasses. We infer from the differences in proportions, but not amounts of urea recycling, that urea metabolism in steers fed CBS vs. the other grasses was controlled more by N supply relative to the steers' nutrient requirements than by morphology (leaf:stem) or N fractions in CBS.

We conclude that the composition of true protein and protein fractions of the 3 grasses were similar, at least in terms of their effect on urea metabolism in steers. Serum urea N concentration and urea N entry rate increased as CP (% of DM) increased among grasses, but differences among grasses in plant morphology, including leaf:stem ratio and CP concentration of leaf and stem, were not reflected in differences in urea production and recycling of urea. This, in part, is due to grazing ruminant's preference for leaf vs. other plant parts. The main distinctions between our work and published work on urea metabolism are that we used grazing animals that selected their diet rather than confined animals, we did not measure intake, and we collected urine for 6 rather than 24 h to measure urine urea N excretion. Those distinctions plus the inherent variabil-

ity in pastures from year to year may explain why we did not detect differences.

LITERATURE CITED

Agbossamey, Y. R., P. Savoie, and J. R. Seoane. 1998. Effect of maceration on nitrogen fractions in hay and silage. Can. J. Anim. Sci. 78:399–405.

Alzueta, C., R. Caballero, A. Rebolé, J. Treviño, and A. Gil. 2001. Crude protein fractions in common vetch fresh forage during pod-filling. J. Anim. Sci. 79:2449–2455.

Archibeque, S. L., J. C. Burns, and G. B. Huntington. 2001. Urea flux in beef steers: Effects of forage species and nitrogen fertilization. J. Anim. Sci. 79:1937–1943.

Archibeque, S. L., J. C. Burns, and G. B. Huntington. 2002. Nitrogen metabolism of beef steers fed endophyte-free tall fescue hay: Effects of ruminally protected methionine supplementation. J. Anim. Sci. 80:1344–1351.

Bach, A., S. Calsamiglia, and M. D. Stern. 1995. Nitrogen metabolism in the rumen. J. Dairy Sci. 88:E9–E21.

Burns, J. C., D. S. Fisher, K. R. Pond, and D. H. Timothy. 1992. Diet characteristics, digesta kinetics, and dry matter intake of steers grazing Eastern Gamagrass. J. Anim. Sci. 70:1251–1261.

Elizalde, J. C., N. R. Merchen, and D. B. Faulkner. 1999. Fractionation of fiber and crude protein in fresh forages during the spring growth. J. Anim. Sci. 77:476–484.

Ellis, W. C., E. M. Bailey, and C. A. Taylor. 1984. A silicone esophageal cannula; its surgical installation and use in research with grazing cattle, sheep and goats. J. Anim. Sci. 59:204–209.

Fisher, D. S., J. C. Burns, K. R. Pond, R. D. Mochrie, and D. H. Timothy. 1991. Effects of grass species on grazing steers: 1. Diet composition and ingestive mastication. J. Anim. Sci. 69:1188–1198.

Hodgson, J., D. A. Clark, and R. J. Mitchell. 1994. Foraging behavior in grazing animals and its impact on plant communities. Pages 796–827 in Forage Quality, Evaluation, and Utilization. G. C. Fahey Jr., L. E Moser, D. R. Mertens, M. Collins, ed. Am. Soc. Agron. Inc., Madison, WI.

Huntington, G. B., and S. L. Archibeque. 1999. Practical aspects of urea and ammonia metabolism in ruminants. Proc. Am. Soc. Anim. Sci., 1999. http://www.asas.org/jas/symposia/proceedings/0939.pdf Accessed Oct. 30, 2002.

Johnson, C. R., B. A. Reiling, P. Mislevy, and M. B. Hall. 2001. Effects of nitrogen fertilization and harvest date on yield, digestibility, fiber, and protein fractions of tropical grasses. J. Anim. Sci. 79:2439–2448.

Kennedy, P. M., and L. P. Milligan. 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: A review. Can. J. Anim. Sci. 60:205–221.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for N fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347–358.

Lobley, G. E., D. M. Bremner, and G. Zuur. 2000. Effects of diet quality on urea fates in sheep as assessed by refined, non-invasive [15N15N]urea kinetics. Br. J. Nutr. 84:459–468.

Magee, K. J. 2004. Nitrogen metabolism of beef steers fed either gamagrass or orchardgrass hay with or without a supplement. MS Thesis North Carolina State Univ.

Marini, J. C., and M. S. Attene-Ramos. 2006. An improved analytical method for the determination of urea nitrogen isotopomers in biological samples utilizing continuous flow isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 20:3736–3740.

Marsh, W. H., B. Fingerhut, and E. Kirsch. 1957. Determination of urea N with the diacetyl method and an automatic dialyzing apparatus. Am. J. Clin. Pathol. 8:681–688.

Nolan, J. V. 1975. Quantitative models of N metabolism in sheep. Pages 416–431 in Dig. Metab. Rumin.: Proc. IV Int. Symp. Rumin. Physiol. I. W. McDonald and A. C. I. Warner, ed. University of New England Publishing Unit, Australia.

St-Pierre, N. R. 2001. Integrating quantitative finding from multiple studies using mixed model methodology. J. Dairy Sci. 84:741–755.